Cannabis terpenes synergistically modulate hippocampal excitability

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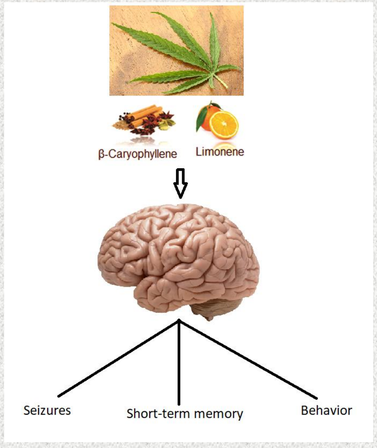
BACKGROUND



- The illicit plant *C. sativa* or "marijuana" contains a multitude of chemicals that contribute to its therapeutic potential.
- The most commonly know chemicals are Tetrahydrocannabinol (THC) and cannabidiol (CBD). CBD has potent anti-seizure properties.
- Cannabis also contains a staggering number aromatic compounds (terpenes) which also have anticonvulsant properties. Terpenes impart the aroma of different plants and are found in common food items.
- We show how terpenes β-caryophyllene (BC) and limonene (LM) effect short-term. Further, combination of LM and BC produced synergistic effects.

HYPOTHESIS

Based on the existing literature on BC and LM, we hypothesize that both will control seizure-like activity and reducing excitability of excitatory neurons.

EXPERIMENTAL DESIGN

All experiments were performed under University of Houston IACUC protocols and SOPs. Hippocampal slices (350µm) were prepared from the brains of 21-24 day old mice. Seizure-like activity was induced using zero magnesium (0 Mg^{2+}) model.

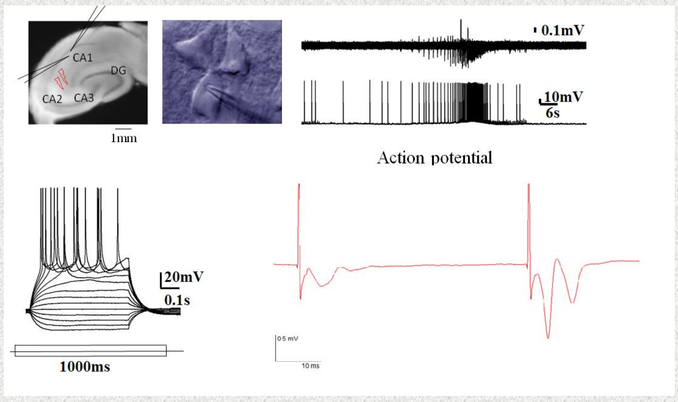
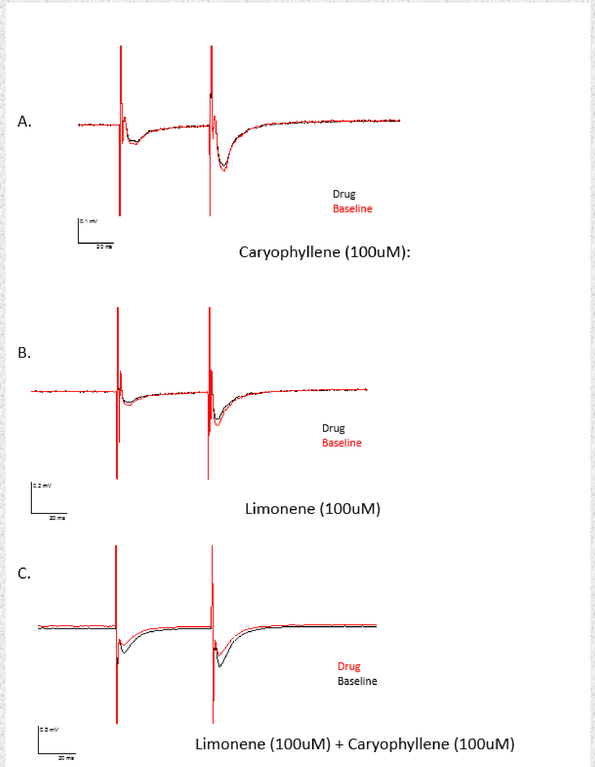


Figure 1. Electrophysiological recordings of network and seizure activity. We placed extracellular (EC) and whole-cell (WC) patch clamp Borosilicate micropipettes in transverse mouse hippocampal slices to record the network and single neuron activity. FEPSPs were induced by CA2 Schaffer collaterals stimulation with concentric bipolar electrodes (200 microns) and FEPSPs were recorded in CA1 stratum radiatum. FEPSPs were induced to observe the effects of terpenes LM and BC on large neuronal population excitability. Paired pulse recordings were performed to observe effects on short term potentiation (STP). In addition, whole-cell voltage clamp recordings and concurrent voltage sensitive dye (VSD) imaging were perform to determine the spatiotemporal pattern of hippocampal circuit activation during seizure conditions; however, this data remains to be analyzed. We used zero magnesium ACSF to induced neuronal hyperexcitability. 2% Dimethyl sulfoxide (DMSO) and 98% deionized water was used to dissolve LM and BC (Sigma).

RESULTS and DISCUSSION

Synergistic depression of hippocampal excitability and PP ratio



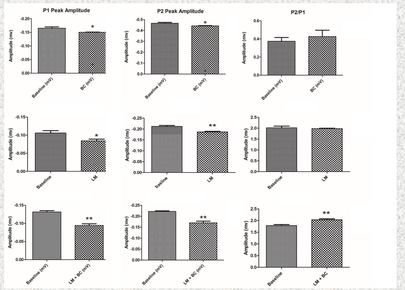


Figure 2. LM and BC individually and synergistically reduce network excitability . (A) The effect of 100 µM BC on paired pulse amplitude. Application of BC significantly reduced the amplitude of both field responses induced by PP stimulation ($P < 0.05$). The ratio of reduction between the second and first (PP ratio) response was not significant. (B) Similarly, 100 µM LM significantly reduced FEPSP amplitude in both the first ($p<0.05$) and second ($p<0.01$) pulse stimulation. Again, no significant difference was observed in the paired pulse ratio. (C) Application of both LM and BC caused greater reduction than either BC or LM alone. Field potential reduction in response the first ($p<0.001$) and second ($p<0.01$) pulse were both significantly reduced. Importantly, combination of both substances increased the paired pulse ratio.

CB_2 agonist BC did not prevent seizure like activity

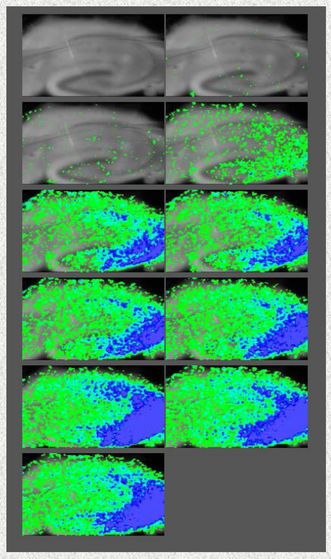


Figure 3 . Electrophysiological recordings of network and seizure activity. **Above:** Whole cell voltage clamp was performed in order to see the effect of BC on seizure initiation. Seizures were induced by perfusion of $0Mg^{2+}$ ACSF and increasing bath temperature to 36ºC. BC did not suppress seizure induction.

Side: In addition, whole-cell voltage clamp recordings and concurrent voltage sensitive dye (VSD) imaging were perform to determine the spatiotemporal pattern of hippocampal circuit activation during seizure conditions; however, this data remains to be analyzed. Zero magnesium ACSF was used to induced neuronal hyperexcitability. Whole cell voltage clamp was performed in order to see the effect of BC on seizure initiation. Seizures were induced by perfusion of 0Mg2+ ACSF and increasing bath temperature to 36ºC.

Discussion

This work demonstrates how commonly ingested aromatic compounds (terpenes, flavonoids, and odorants) can induce physiological changes on the molecular level in brain regions implicated in high order information processing. Indeed, the component discussed in this study (BCP) is found in relatively high concentrations in common household spices such as cloves and black pepper. Also, limonene is commonly found in the rinds of citrus fruits (e.g. lemon and oranges). These physiological actions could be responsible for individual preferences of different types of foods and aromas that act synergistically with our bodies and modify behavior.

Further, this work provides tentative evidence for the entourage hypothesis. It is worth noting that the synergistic actions of LM and BC not only caused a greater reduction in network excitability, but it also reduced paired pulse facilitation. Network facilitation is a necessary component for short term and long term memory. Our study would seem to indicate that BC and LM could potentially effect short term memory. Whether this effect is due to the combined actions of CB_2 (BC) and GABA (LM) receptor activation or some other mechanism remains to be discovered. In addition, the role of the CB_2 receptor in the central nervous system and information procession is hotly debated; however, accumulating evidence support its role in higher brain processes. We have indicated that combination of different components may result in emergent neuro-modulatory capabilities; however, further studies are required to confirm these findings.

Lastly, the need for effective treatments for severe pharmacoresistant forms of pediatric epilepsies such as Dravet syndrome is of imminent importance. Due to current drug laws, it is legally and financially impractical for many people to obtain high quality and high concentration C. sativa extracts. This could lead to ingestion of inferior products with chemical contamination or highly variable concentrations of chemical components. This issue may be completely circumvented by using natural non-cannabis derived cannabinoid agonists. The only limitation is our lack of knowledge and testing regarding these substances.

Future Directions

It is still not know whether BC and LM combination is capable of controlling seizure initiation, however, this study indicates that BC by its self does not. Further the mechanisms by which these molecules mediate their actions completely unknown. I would like to test pre- and post- synaptic potentials to zone in on the systems responsible. A substantial amount of research is still required elucidate if and how BC, LM, other terpenes and cannabinoid affect excitability in the Dravet Model.

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Acknowledgments

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